Vegetative Reproduction in the Ferns I. Leaf Buds of Grammitis tenella

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Morphogenetic studies have shown that the developmental response of ferns to experimental treatment is very broad (see Cutter, 1965). Particularly the early surgical experiments on intact plants (Wardlaw, 1952) indicated how plastic the meristematic areas are. This work has become the basis of more refined experiments, including sterile culture and microsurgical techniques. One of the more recent experiments on intact plants showed that even cells of the petiole of *Dryopteris*, which were presumably mature and fully differentiated, could be caused to become meristematic (Cutter and Wardlaw, 1963). Surgical isolation of patches of cells permitted new meristems to develop and eventually well organized plantlets formed on the petioles of the experimental plants. The natural occurrence of vegetative buds on the various organs of the fern sporophyte is well known (McVeigh, 1937; White, 1968).

While sampling young fern sporophytes for comparative studies of the vascular tissue, I found several leaves of *Grammitis tenella* Kaulf. which had become detached from the parent plant, touched the soil, and developed outgrowths from one surface. These outgrowths, which were in various stages of development, are capable of forming new plants. The development of these buds on mature leaves is unusual and is a new instance of vegetative reproduction for the ferns.

MATERIALS AND METHODS

I collected spores of *Grammitis tenella* on a field trip to the Hawaiian Islands in the summer of 1964. In the laboratory, the spores were surface sterilized in a 10% sodium hypochlorite solution, rinsed in sterile distilled water, and sown, under sterile conditions, on nutrient agar in petri dishes. After the initiation

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of young sporophytes, the agar with the sporophytes was transferred to soil in pots. The pots were placed in the greenhouse on benches over which cloth screens had been placed to reduce light; an automatic water spray system provided a fine mist which maintained a high humidity level around the plants. Some 200 species of ferns have been grown successfully with this technique.

Pictures of the unusual leaves were taken with a single lens reflex camera prior to preparing them for microscopic study. The specimens were killed and fixed in Navashin solution (CRAF), embedded, sectioned, stained, and prepared for microscopic study in the usual manner. Stained sections were photographed with a 35 mm Leitz Ortholux camera. For comparative purposes, leaves of a normal plant and the thallus of a normal *Grammitis* gametophyte were sectioned and stained.

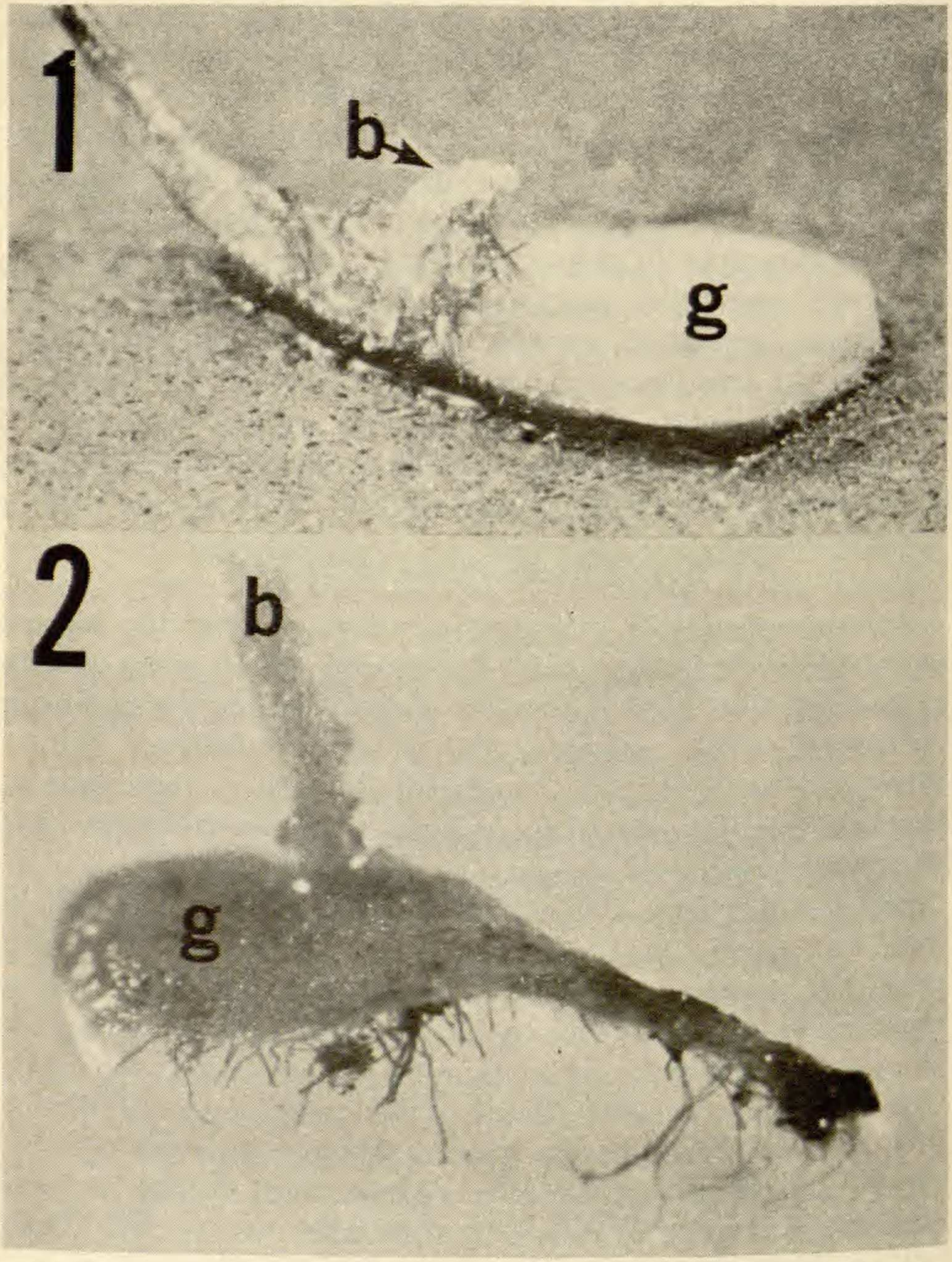
NORMAL GAMETOPHYTES AND LEAVES

Because vegetative reproduction might replace the normal sexual life cycle in this plant, I sectioned several gametophytes to confirm that both antheridia and archegonia occur. Water mounts of living gametophytes revealed swimming sperms. Finally, sporophytes were found still attached to their gametophytes. Sexually-produced sporophytes were initiated 40–60 days after spores were sown on agar, and developed normally.

The leaf anatomy of a normal plant of *Grammi is tenella* is that of a typical fern. Compact palisade parenchyma and spongy parenchyma with numerous air spaces compose the bulk of these small leaves. Both leaf surfaces have a distinct epidermis, and the lower leaf surface typically contains more stomata than the upper. One main vein extends the length of the leaf; small laterals branch from it at intervals. In the upper epidermis bulliform cells are usually associated with the vein endings. Leaf curling in a dry atmosphere is probably caused by these cells.

LEAF OUTGROWTHS

Twelve samples of leaf buds of *Grammitis tenella* in various stages of development were prepared for study. The outgrowths



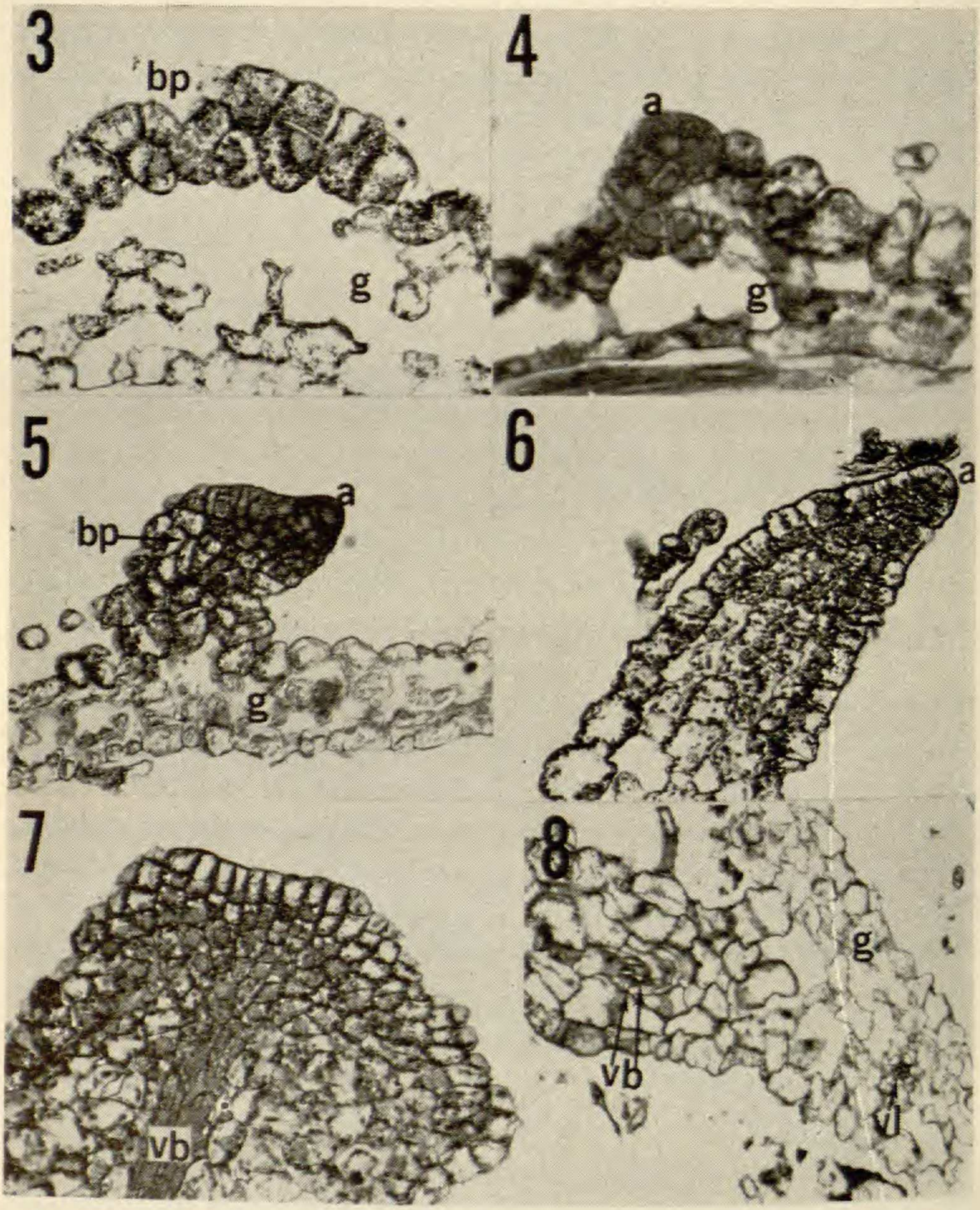
Adventitious Buds from Lower Surface of Grammitis Leaf. Fig. 1. Outgrowth with stem morphology, ×5. Fig. 2. Typical outgrowth with leaf morphology, ×5.

always occur on the lower (abaxial) leaf surface, which is the one with the most stomata, the one most closely associated with the spongy parenchyma tissue, and the one toward which the phloem is found in the main vein as seen in cross section of the leaf (*Figs. 1* and *2*). When leaves fall from the plant, buds develop only in those that land with the abaxial leaf surface up. No buds grew into the soil from the adaxial leaf surface, nor when the abaxial surface was down.

Cell divisions first occur in the previously mature epidermal cells of the adaxial leaf surface. Rhizoids develop and are cut off from the epidermal cells by cell walls. Hairs or scales also may develop from previously mature epidermal cells surrounding the area in which the bud will form.

Initiation and development of the outgrowth continues with divisions of the parenchyma cells just below the abaxial epidermal cells (*Fig. 3*). The epidermis bulges at first, but subsequent development involves the epidermal cells, for no mechanical breaks occur in this layer (*Figs. 4* and *5*). As cell divisions continue, the meristematic mass becomes three dimensional and rounded (*Fig. 5*), and usually develops into a leaf primordium (*Fig. 6*). The single apical cell and its orientation to the surrounding cells of the primordium is similar to that found in many young fern leaves. By continued division and elongation of the meristematic cells, the outgrowth increases in size considerably (*Figs. 6* and *7*). Following the initial increase in size, typical leaf flattening occurs. Stomata are present on both surfaces. The venation pattern in paradermal section is typical of mature *Grammitis* leaves (*Fig. 7*).

Very young primordia are composed of solid parenchyma; there is no evidence of provascular tissue. Following the formation of a distinct apical cell, however, provascular tissue differentiates (*Fig. 7*). Cells just above the connection between the outgrowth and the parent leaf surface divide and stain differentially. As development of the new leaf continues, a vascular strand extends upward toward the primordium tip from an early-formed vascular nodule in the base of the leaf (*Fig. 8*). Vascular differentiation



Histological Detail of Leaf Bud Development. Fig. 3. Origin of outgrowth in superficial parenchyma of abaxial leaf surface, ×195. Fig. 4. Apical organization in young primordium, ×190. Fig. 5. Bud primordium with well-organized meristem, ×95. Fig. 6. Median longisection of outgrowth with typical apical cell, epidermis and parenchyma, ×190. Fig. 7. Paradermal section of outgrowth through parenchyma and vascular strand, ×115. Fig. 8. Attachment of out-

occurs in an acropetal direction from this nodule toward the leaf tip. There is no connection between the vascular system of the outgrowth and that of the parent leaf (*Fig. 8*). As the new outgrowth continues to grow and elongate, cells at the base of the outgrowth become meristematic. Usually these cells are located where the outgrowth connects to the abaxial leaf surface. A bud primordium originates by divisions in the parenchyma surrounding the vascular strand (*Figs. 9* and *10*). The initiation of cell divisions here causes renewed cell division in surrounding cells, and the primordium increases in size (*Fig. 11*). An apical meristem is organized just before the bud emerges from the surface of the outgrowth. Apical cell morphology is typical of the shoot apices of most polypodiaceous ferns and is very similar to that found in the *Grammitis* sporophyte (*Figs. 11–13*). After a shoot meristem is organized root initials differentiate in the bud primordium (*Fig. 13*). As in the development of lateral roots on most fern stems, each root develops endogenously in shoot tissue, and generally at least one root is associated with each leaf that is produced at the shoot apex.

Following the development of a functional apical meristem, internal vascular tissue differentiation takes place. In contrast to the lack of vascular continuity between the outgrowth and the parent leaf, the shoot meristem is connected with the vascular strand of the outgrowth (*Figs. 9*, *10*, and *14*). Although the first provascular tissue develops in the newly formed shoot meristem area, further differentiation of xylem is acropetal and proceeds from the vascular strand of the outgrowth into the meristem of the new shoot primordium. A single vascular strand connects the

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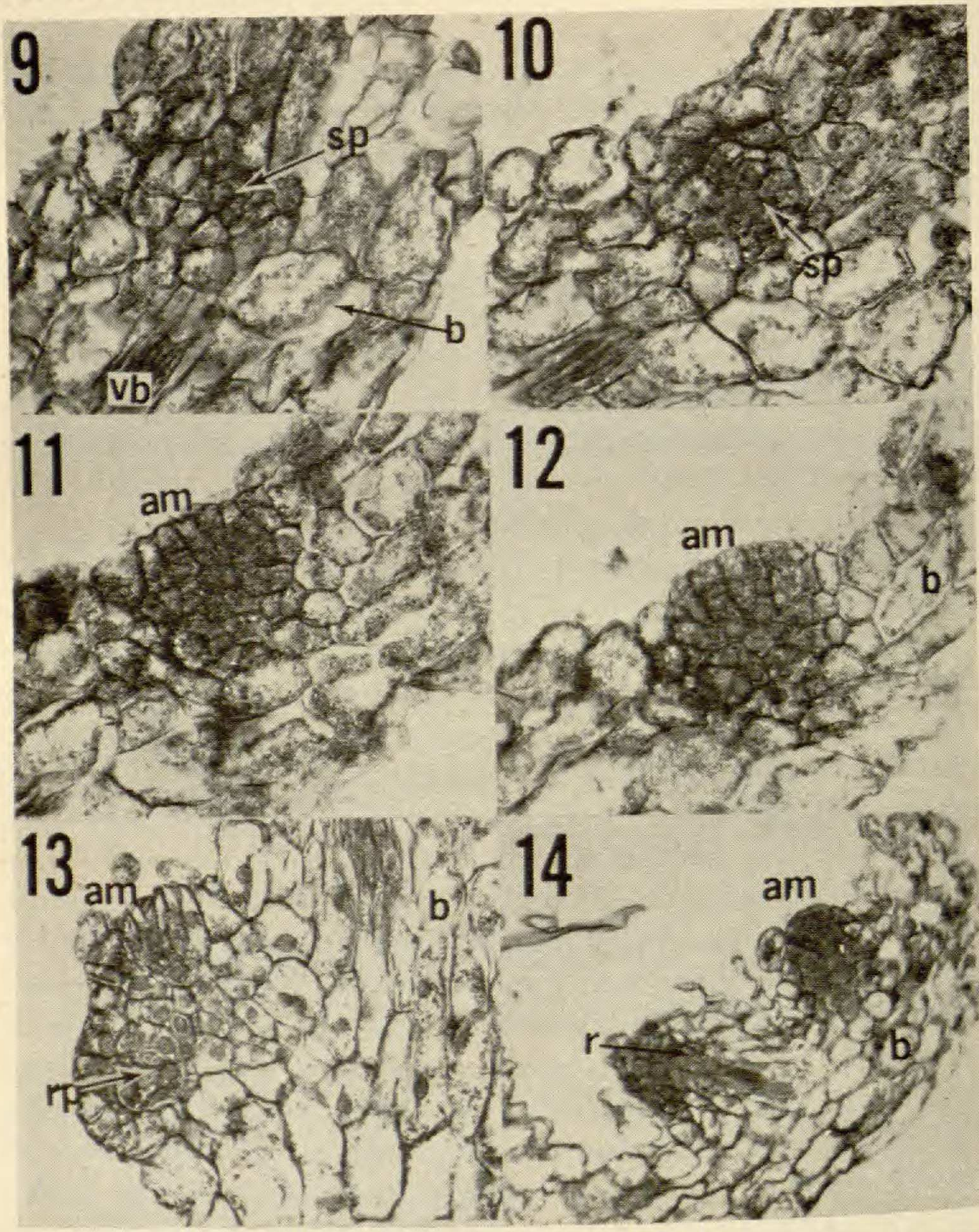
GROWTH AND LEAF WITH VASCULAR STRAND OF OUTGROWTH AND VEIN OF PARENT LEAF UNCONNECTED, ×115. The abbreviations are: *a*, APICAL CELL; *am*, APICAL MERISTEM OF SHOOT; *b*, ADVENTITIOUS BUD; *bp*, ADVENTITIOUS BUD PRIMORDIUM; *g*, GRAMMITIS LEAF; *r*, ROOT; *rp*, ROOT PRIMORDIUM; *sp*, SHOOT PRIMORDIUM; *vb*, VASCULAR TISSUE OF BUD; *vl*, VEIN OF GRAMMITIS LEAF.



Histological Detail of Shoot Formation. Fig. 9. Origin of new shoot primordium in parenchyma associated with vascular tissue of the adventitious outgrowth, ×680. Fig. 10. Additional mitotic divisions in shoot primordium, ×200. Fig. 11. Apical meristem of shoot primordium, ×200. Fig. 12. Development of root primordial initials, ×200. Fig. 13. Distinct apical and root meristems on endogenous bud, ×200. Fig. 14. Organized adventitious bud with shoot and root meristems, ×100. The abbreviations are identical to Plate 18.

vascular tissue of the leaf outgrowth to that of the developing bud.

One sample differed in its later stages from the pattern of development described above. In contrast to the others it developed and maintained radial symmetry, and morphologically appeared to be a stem rather than a leaf. In addition to radial symmetry, there were few stomata present on the surface of the outgrowth and there was a simple, single, central vascular strand. Careful analysis of the vascular strand revealed mesarch xylem differentiation. At the tip of this stem-like outgrowth, a flange of tissue differentiated which had obvious leaf-like characters: stomata primarily on one surface, dorsiventral flattening, a simple large terminal apical cell, and branched venation. Groups of cells which stained differentially occurred where the leaf-like flange was attached to the radially symmetrical outgrowth. These meristematic cells developed into a bud primordium similar to those described previously which occur where the outgrowth is attached to the parent leaf. As in the other samples, a simple vascular strand connected the meristematic area with the vascular strand of the outgrowth. In addition to the hairs or scales which had developed from the parent leaf surface, appendages similar to root hairs developed from the surface cells of the outgrowth at the point of attachment to the parent leaf.

DISCUSSION

The development of buds in *Grammitis tenella* from the abaxial surface of mature leaves which have come in contact with soil or other suitable substrate appears to be unique in the ferns. In most instances of plantlet formation from fern leaves, bulbils are present on the leaves of intact plants (Marchal, 1965) or the culture conditions are more harsh (Morlong, 1967). Gametophytic outgrowths occur in other cases of detached fern leaves growing under conditions similar to those described for these leaves.

Development of leaf outgrowths in *Grammitis* involves considerable dedifferentiation of mature parenchyma cells. Mature cells which compose the spongy mesophyll of the mature leaf first become meristematic. Epidermal cells of the adaxial leaf surface

undergo renewed cell division to form rhizoid-like structures similar to those found on typical fern gametophytes. Epidermal cells of the abaxial leaf surface also undergo renewed cell division and form scales and hairs which surround the developing bud.

On the basis of the developmental stages noted in this study, the initial outgrowth from the mature sporophytic leaf is usually a leaf. The apical organization, dorsiventral flattening, stomatal arrangement, and vascular pattern of these outgrowths support this conclusion. Interestingly, vascular differentiation in the outgrowth does not occur until a typical leaf apical cell is formed. Subsequent to the formation of the apical cell, xylem differentiation begins in the outgrowth and appears to proceed under its control. In contrast to previous reports of adventitious buds on other fern leaves (Marchal, 1965), in *G. tenella* no connection is ever made between the vascular strand of the parent leaf and the vascular tissue of the lateral outgrowth.

Organized stem and root meristems develop after the apical cell of the leaf-like outgrowth has formed. Thus, in addition to vascular differentiation, the initial organization of the new shoot meristem at the base of the outgrowth probably is under the control of the apical cell also. The morphology and development of the shoot meristem, which is at the juncture of the outgrowth with the parent leaf, is very similar to that of a typical axillary bud. The histogenesis of this bud is very similar to that of many normal axillary buds in other ferns (e.g. *Marsilea*, White, 1966). Eventually it forms young leaf primordia in addition to the main stem apex, and one to several young adventitious root primordia may form also. Since the location of the meristem on the outgrowth is not in the leaf axil, it is not truly an axillary bud.

In one sample, the morphology of the outgrowth indicates it was basically a stem. The organ was radially symmetrical, had few stomata, and the xylem differentiation pattern in the axis was mesarch. Interestingly, the terminal portion of this outgrowth developed a flattened flange of tissue which was leaf-like in its venation pattern, stomatal arrangement, and terminal meristem organization. In this case, the new shoot meristem differentiated

at the juncture of the flange with the radially symmetrical outgrowth. If the radially symmetrical outgrowth is a stem, this one case would be an example of true axillary bud formation. The possibility cannot be discounted, however, that the radially symmetrical outgrowth is a leaf petiole, with the flange equivalent to a leaf blade. The distance of the new shoot meristem from the juncture of the outgrowth and the parent leaf was different for each of the samples. This is somewhat similar to that described by Wardlaw (1949) for "detached meristems" with reference to the variation in location of normal axillary buds. If the radial outgrowth is a petiole rather than a stem, the shoot meristem is not an axillary bud. It would be merely a displaced meristem and, except for its location, similar to the other samples described here.

Based on all the data, the simplest explanation is that a primordial leaf develops from the abaxial surface of a detached mature sporophyte leaf. The vasculature of this appendage and a new shoot meristem are both differentiated under the influence of the apical cell of this developing leaf. The shoot meristem, although not axillary to a stem, has all the morphological and developmental characteristics of a true axillary bud.

In the one case where an outgrowth grew successfully to maturity, the initial leaf-like outgrowth did not develop substantially, but rather the shoot meristem continued to grow and develop additional young leaves and roots. Eventually the parent leaf shrivelled and disintegrated, while the new plant grew to maturity and bore viable spores.

In the dense, rainy areas of Hawaii where these plants were collected, it is conceivable that such a method of asexual reproduction could be of some benefit to the plant. Although obviously not contributing to genetic variability, it would establish populations of the species. My general collections of the fern were always of large clusters of plants of various ages. A careful survey of four large preserved collections of this species from two trips to Hawaii revealed only six leaves that had small flanges on them. The leaf outgrowths generally are very fragile, and several of those found in the greenhouse pots were destroyed by rough handling. My col-

lections usually were of the larger, more easily seen plants, and were pressed tightly into bottles of FAA, possibly precluding the preservation of very many useful or recognizable outgrowths. The leaf budding phenomenon that I found in the laboratory may occur in nature, but more careful observations and field collections are called for.

The natural development of whole young plants from previously mature and differentiated cells adds further support to the results of experimental studies previously carried out on intact plants (Wardlaw, 1952; Cutter and Wardlaw, 1963). The potentials for differentiation and development that have been elucidated through experimental morphological techniques appear in this instance to have been realized in nature also. The problem of the physiological control of the development of these leaf outgrowths in *Grammitis* remains to be solved. A preliminary investigation into this question is to begin shortly (C. W. Smith, Univ. of Hawaii, pers. comm.).

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